Jurnal Teknologi

Full Paper

INHIBITORY EFFECT OF *ARTOCARPUS LOWII* KING COMPOUNDS ON COX-2 AND 15-LO ACTIVITIES

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Article history
Received
7 November 2014
Received in revised form
30 March 2015
Accepted
1 August 2015

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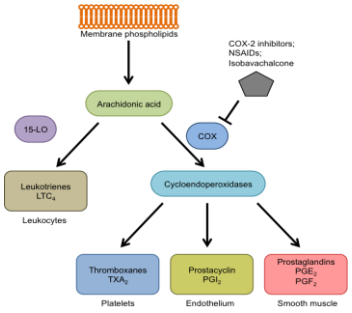
Graphical abstract



Abstract

Artocarpus lowii King is a rare species of plant in Moraceae family. In this study, the anti-inflammatory activity of cycloheterophyllin, isobavachalcone, 4-hydroxylonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone isolated from *A. lowii* King were investigated on classical enzymes in arachidonic acid metabolism pathways; cyclooxygenase and lipoxygenase. Isobavachalcone directly inhibited cyclooxygenase-2 enzyme in dose dependent manner, with IC_{50} value of 0.95 µM. No noticeable effect has been observed with the other tested compounds. In addition, none of the tested compounds displays a direct inhibition on 15-lipoxygenase when compared to the resveratrol as control with the IC_{50} value of 1.5 µM. Isobavachalcone showed inhibitory effect on cyclooxygenase-2. This study suggests that *A. lowii* King contains potential anti-inflammatory activity.

Keywords: *Artocarpus lowii* King, Anti-inflammatory, Cyclooxygenase-2, Isobavachalcone

Abstrak

Artocarpus lowii King adalah sejenis spesis tumbuhan jarang daripada keluarga Moraceae. Dalam kajian ini, efek anti-inflammatori oleh komponen aktif daripada *A. lowii* King iaitu cycloheterophyllin, isobavachalcone, 4-hydroxylonchocarpin dan 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone ke atas aktiviti enzim cyclooxygenase-2 dan 15-lipoxygenase telah diselidiki. Isobavachalcone secara lansung telah menghalang aktiviti cyclooxygenase-2 dengan nilai IC_{50} 0.95 µM. Tiada aktiviti memberangsangkan untuk kompaunn lain. Selain itu, tiada aktiviti direkodkan untuk efek terhadap 15-lipoxygenase bagi semua kompaun. Hanya kompaun control iaitu resveratrol menunjukkan aktiviti dengan nilai IC50 sebanyak 1.5 µM. Isobavachalcone dicadangkan memiliki efek anti-inflammatori yang menyumbang kepada aktiviti anti-inflammaotori sepsis *A. lowii* King.

Kata kunci: *Artocarpus lowii* King, Anti-inflammatori, Cyclooxygenase-2, Isobavachalcone

1.0 INTRODUCTION

Globalization has driven a remarkable transformation in living aspect relating to lifestyle, work-nature, and dietary habits. The reflection of this setting can be seen in the rise of degenerative diseases cases in population around the world [1]. The diseases such as cardiovascular diseases (CVD), hypertension, diabetes and obesity have become prominent over the past few years [2]. Study conducted by the International Medical Foundation of Japan revealed that the cases of CVD increased drastically since 1965 until 1997 and the number of cases is approaching alarming state. Recent preliminary report by the World Health Organization (WHO) showed that heart related diseases are the topmost leading causes of death in United States [3]. This situation has alerted medical practitioners and scientists to undergo pathophysiological and therapeutic approaches in heart-related diseases.

Natural products such as coumarin and aspirin are great sources of traditional and modern drugs for treatment of different heart-related diseases [4]. According to the WHO, approximately 80% of the world's populations rely on traditional medicine for their primary health care [5]. Folks applied different plants extracts for treatment of wide variety of diseases including chronic inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that are capable to provide analgesic, antipyretic and anti-inflammatory effects [6]. Even though NSAIDs are currently used in the treatment of inflammation, these drugs have not shown any significant effect in treating chronic inflammatory disorders. In contrast, several compounds are found to be associated with negative side effects. Thus, there is a need to search for non-poisonous and effective anti-inflammatory compounds [7].

Phytochemicals are reported to be key players in promoting various medicinal activities. Phytochemicals such as flavonoids have demonstrated numerous biological activities such as anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities [8-10]. This type of compounds was also reported to exhibit *in vitro* and *in vivo* anti-inflammatory activities in mice [11-12]. Cohort studies demonstrated that an increased in the consumption of plant-derived foods like fruit and vegetables, nuts, and whole grains is associated with a reduced risk of heart-related diseases [13]. Therefore, flavonoids are presumed to be vital in promoting anti-inflammatory activities, and also in developing a new kind of anti-inflammatory agents [14]. In addition, wide attention of natural-derived medicine occurs probably due to the fact that it possesses less adverse compared to the synthetic counterpart. Natural products also are basically safe and easily obtained.

Artocarpus lowii King is a scarce species of tree in the Artocarpus genus of the Moraceae family. This plant is widely distributed in South East Asia. In Malaysia, it is locally known as 'miku' while in is known as 'bangsal' in Indonesia [15]. Even though there are numerous biological reports on Artocarpus genus, there is little study on anti-inflammatory of *A. lowii* King. Flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis* had shown anti-inflammatory activities through inhibition of superoxide anion formation in fMLP-stimulated (Formyl-Methionyl-Leucyl-Phenylalanine) rat neutrophils and the stimulation of superoxide anion generation [16].

We report herein the investigation on the anti-inflammatory activity of isolated compounds from the leaves of *A. lowii* King on the cyclooxygenase-2 and 15-lipoxygenase enzyme pathways.

2.0 EXPERIMENTAL

2.1 Sample Preparation

Cycloheterophyllin, isobavachalcone, 4-hydroylonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone were provided by Jamil *et al*. [15]. Resveratrol served as positive control, the samples were dissolved in Ethyl Acetate (EtOAc) and diluted ten-fold into five different concentration ranges from 0 μM to 100 μM. All samples were stored at 4 °C to avoid decomposition.

2.2 COX-2 Inhibitor Screening Assay Kit

COX-2 inhibitor screening kit (Item No.: 560131; Cayman Chemicals, USA) was used. The assay included two major steps, COX reaction and enzyme immunoassay. In COX reaction, the required reactions such as background reaction, initial activity reaction, and sample reaction were undergone in 100 mM Tris-HCL buffer containing 1 μM heme and COX-2 (human recombinant). The reactions were pre-incubated for 10 minutes at 37 °C. The reaction was later introduced with 10 μL of arachidonic acid. After 2 minutes, an amount of 1 M of HCL was added into each sample to terminate the reaction process. Reactions were transferred into 96 well plate coated with mouse anti-rabbit immunoglobin (IgG). The tracer, prostaglandin acetylcholine esterase, and primary antibody were then inserted into each well. After 24 hrs of incubation at room temperature, the reaction mixtures were removed and all wells were washed with 10 mM potassium phosphate buffer containing 0.05% Tween 20 solution. Then, Ellman's Reagent was added into wells. The plate was incubated for approximately 60 minutes until the maximum binding wells (control) reach the optical density (OD) ranging between 0.3-0.8 at λ_{415nm}.

The acquired raw data from plate reader were calculated by applying the data into the equation provided in the manufacturer's protocol below.

$$\text{Percentage of Inhibition (\%)} = \frac{[\text{Total COX activity}]-[\text{Total COX activity (inhibitor)}]}{[\text{Total COX activity}]} \times 100\%$$

The IC_{50} value is presented; as the value is the amount of inhibitor required to disrupt the enzyme activity by 50%.

2.3 15-Lipoxygenase Inhibitor Assay

Lipoxygenase inhibitor screening assay kit (Item No. 760700) was purchased from Cayman Chemical, USA. All required reagents were prepared according to the provided protocol. An amount of 100 µl of Assay Buffer was added into three wells. In positive control wells (15-LO standard), 90 µl of 15-LO and 10 µL of Assay Buffer were added. Approximately, 90 µl of lipoxygenase enzyme and 10 µl of Ethyl Acetate were inserted to at least two wells (100% Initial Activity wells). An amount of 90 µl of lipoxygenase and 10 µl of inhibitor were added into inhibitor wells. In this experiment, only cycloheterophyllin and isobavachalcone were studied as inhibitors together with the control (resveratrol) on 15-lipoxygenase (15-LO) activity. The wells were incubated for five minutes at room temperature. The reaction was initiated when 10 µL of linoleic acid was added into the wells. Approximately 100 µL of chromogen was pipetted to stop the reaction. The reactions were measured at 515 nm. Percentage of inhibition of sample was calculated by following the equation below as instructed in the manufacturer's protocol:

$$\text{Percentage of inhibition (\%)} = \frac{100\% \text{ Initial activity wells} - \text{Inhibitor wells (sample)}}{100\ \% \text{ Initial Activity wells}} \times 100$$

3.0 RESULTS AND DISCUSSION

3.1 Compounds Effect On COX-2 Inflammatory Mechanism

As shown in Figure 1 below, isobavachalcone showed a significant dose-dependent inhibitory effect on COX-2 activity with IC_{50} value of 0.95 µM. Meanwhile 4-hydroxylonchocarpin displayed a reverse effect on COX-2 activity. This compound exhibited higher inhibition at lower concentration with 59.18% at 0.1 µM. The effect gradually decreased to 27.1% at 10 µM and rose slightly 28.19% at 100 µM. Resveratrol (control) have a steady effect on COX-2 activity even though at 100 µM, the data showed an outlier result.

Isobavachalcone was reported to demonstrate biological activities such as anticancer, antibacterial, antiplatelet, antifungal and anti-tubercular [17-19]. Shin *et al*. [20] reported that isobavachalcone isolated from *Angelica keiskei* restrained inducible nitric oxide synthase (iNOS) enzymes expression thus deterring the inflammatory substance, nitric oxide (NO) production. However, the mechanism of action exerted by isobavachalcone remains uncertain. The current study demonstrated that isobavachalcone directly inhibit COX 2 enzyme.

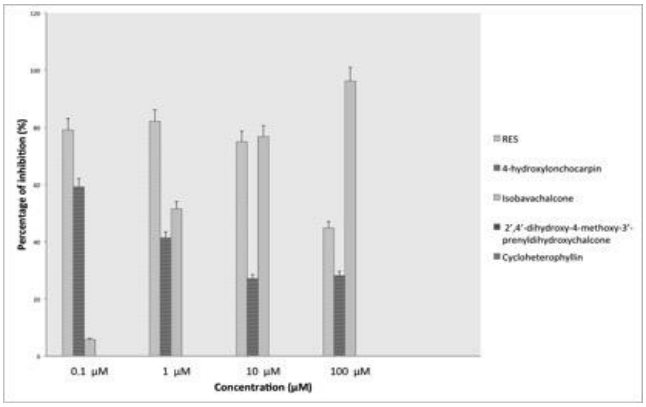


Figure 1 Inhibitory effects of compounds with resveratrol (RES) as control on cyclooxygenase-2 activity

3.2 15-Lipoxygenase Inhibition Activities

In contrast, no inhibitory effect observed on all investigated compounds except for resveratrol (control). In Figure 2, resveratrol has displayed a significant inhibitive activity on 15-lipoxygenase. Cycloheterophyllin observed in this study is contrary to the one isolated form *Artocarpus heterophyllus* LAMK inhibited arachidonate 15-lipoxygenase [21]. We presumed the dissimilar in structure of linoleic acid and arachidonic acid might produce this significant results. Linoleic acid structure contains less double bond compared to arachidonic acid hence the sensitivity of arachidonic acid is higher.

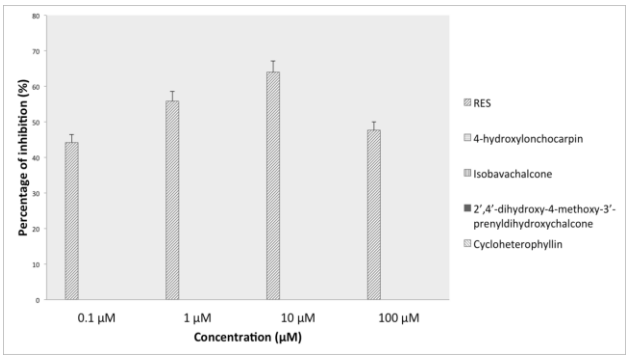


Figure 2 Inhibitory effects of compounds with resveratrol (RES) as control on 15-lipoxygenase activities.

4.0 CONCLUSION

This study shows that isobavachalcone isolated from *A. lowii* King possess potential anti-inflammatory properties via direct COX-2 inhibitory mechanism but not 15-LO. However, no inhibitory effect on both COX-2 and 15-LO by cycloheterophyllin, 4-hydroxylonchocarpin and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydroxychalcone isolated from *A. lowii* King.

Acknowledgement

The authors would like to acknowledge to Research University Grant (GUP) from the Ministry of Higher Education (MOHE) for financial support under vote 01G59 and 07H22 and the Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia for facilities supports.

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